ADDITION OF THIOGLYCOLIC ACID TO STENDOMYCIN

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Base-catalyzed addition of thioglycolic acid to the dehydrobutyrine residue in stendomycin followed by hydrolysis and isolation of the newly formed amino acid (S-carboxymethyl-β-methylcysteine) provided a pair of optically active diastereoisomers. Thus, the addition of the thiol to the double bond is stereoselective and is governed by the architecture of the molecule of the antibiotic.

The primary structure of the antifungal antibiotic stendomycin[1] was elucidated in this Laboratory.[2] The proposed sequence of a fatty acid and 14 amino acid residues (Fig. 1) was corroborated by studies[3] of the mass spectra of the permethylated open chain acid prepared by hydrolytic opening of the lactone ring of the antibiotic. A preferred conformation with a folding of the "side chain" over the ring portion of the molecule was suggested[4] by the chemical and physical properties of stendomycin. The occurrence of a dehydrobutyrine (dehydro-α-aminobutyric acid) residue[5] in stendomycin prompted an investigation concerning the possibility of stereoselective addition of thioglycolic acid to the double bond of this moiety. Addition of thiols to dehydroamino acid-containing antibiotics was reported by GROSS and MORELL,[6,7] but without regard to the steric course of the reaction.

Stendomycin was treated with thioglycolic acid in ethanol; the reaction mixture was made slightly alkaline by the addition of piperidine. The progress of the reaction was monitored by amino acid analysis of hydrolysates of samples taken from time to time. When no further increase in the amount of S-carboxymethyl-β-methylcysteine (2-amino-3-carboxymethylmercaptopropionic acid, **I**, Fig. 2) was noted, the solvent was removed and the residue hydrolyzed with 6 N hydrochloric acid. An aqueous solution of the hydrolysate was extracted with ether and passed through a column of the anion-exchange resin Dowex-1, in acetate cycle. Since stendo-

Fig. 1. This structure represents the dominant compound of the stendomycin family. In other members of the stendomycin group, isomyristic acid is replaced by its lower homologs and alloisoleucine by valine or leucine. Δ-But=dehydrobutyrine, Ste=stendomycidine

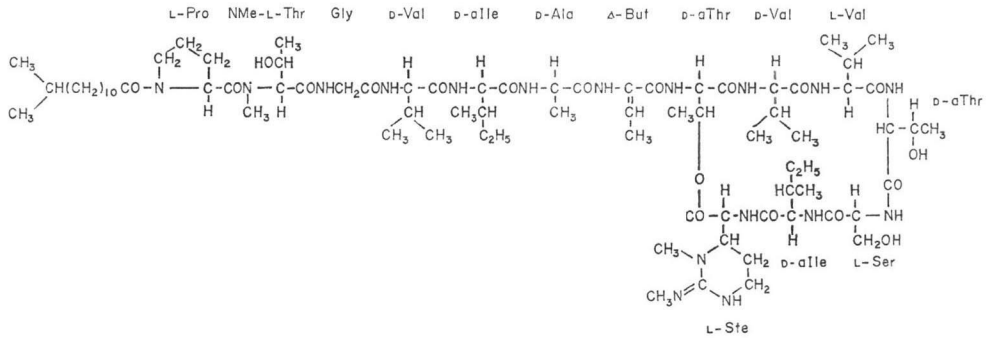
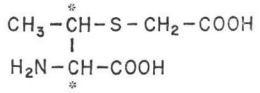
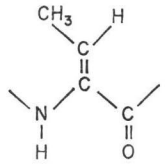


Fig. 2. S-Carboxymethyl -β-methylcysteine.
Centers of asymmetry are indicated by*.



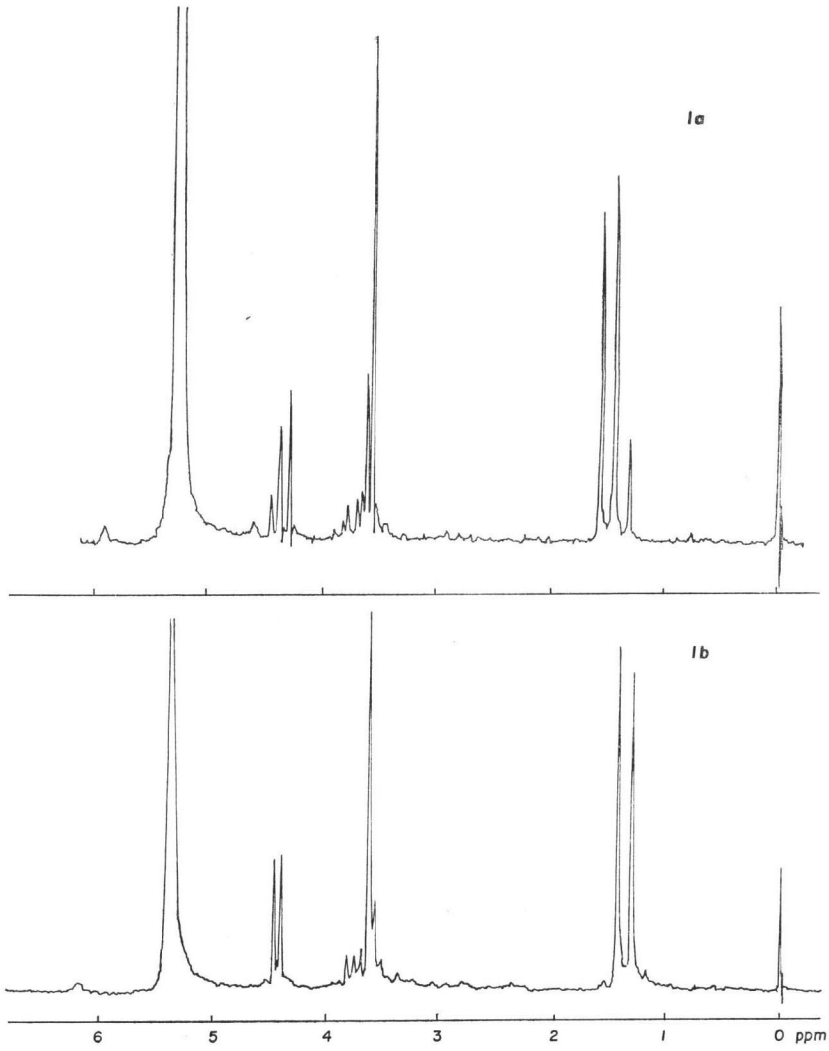
I

Fig. 3. The planar dehydrobutyrine residue.



mycin itself does not contain acidic residues, only the newly formed amino acid was retained by the column from which it was subsequently eluted with dilute acetic acid. Evaporation and trituration of the residue with ethanol afforded a less soluble **Ia** and a more soluble form, **Ib**. The two compounds appear at the same elution volume in amino acid analysis under the conditions developed by SPACKMAN, STEIN and MOORE[8], but they can be distinguished from each other by their nmr spectra in 2 N DCl: *e.g.*, the doublet of the methyl protons of **Ia** appears lower field (by 0.13 ppm) than the corresponding signal of **Ib**, *etc.* Both forms are optically active, although probably not optically pure. In the absence of interferring stereospecific factors, the addition of a mercaptane to the planar dehydrobutyrine

Fig. 4. 60 MHz nmr spectra of **Ia** and **Ib** in N DCl with DDS as internal reference.



residue (Fig. 3) should result in the formation of two diastereoisomeric pairs, the optically inactive racemates of **I**. The optical activity of the isolated diasteroisomers, however, reveals preferential addition of the thiol on a less hindered side. A reasonable explanation is provided by the assumption of a preferred conformation of stendomycin in the reaction mixture. Enhancement of conformational restrictions by ethanol was shown earlier.[4] A well-defined molecular architecture is the most likely cause of the preferential addition of thioglycolic acid from one side of the plane of the dehydrobutyrine residue.

Experimental

Addition of thioglycolic acid to stendomycin and isolation of two forms of S-carboxymethyl-β-methylcysteine

A sample (3.0 g) of the antibiotic was dissolved in 50 % aqueous ethanol (200 ml). Thioglycolic acid (3.7 g) and piperidine (4.5 ml) were added and the clear solution (pH 8.5) was left to stand at room temperature. Four days later,* the solvent was removed by a stream of nitrogen, the residue dissolved in 6 N HCl (200 ml) and boiled under reflux for 24 hours. The solution was concentrated (N_2 stream, steam bath) to about 50 ml, water (100 ml) was added and the mixture extracted with ether (100 ml in two portions). The solution was evaporated to about 30 ml and applied to a column (4 cm×22 cm) of Dowex 1-8X resin in acetate form. The column was washed with water (1,500 ml), 0.2 N acetic acid (500 ml) and eluted with 2 N acetic acid (3×500 ml).

The second fraction contained the bulk of **I**. It was evaporated to dryness and the residue triturated with 95 % ethanol. The insoluble material (114 mg) consisted mostly of **Ia**. The nmr spectrum of crude **Ia**, still containing a small amount of the diastereoisomer **Ib**, is shown in Fig. 4a. A sample (30 mg) was dissolved in hot H_2O (2 ml) and the solution diluted with absolute ethanol (4 ml). The crystals of **Ia** were collected and dried (18 mg); mp 186°C (FISHER-JOHNS block); $[\alpha]_D^{25}+8°$ (*c* 4, 2 N HCl).

Anal. Calcd. for $C_6H_{11}NO_4S$: C, 37.30; H, 5.74; N, 7.25; S, 16.60.
Found: C, 37.55; H, 5.82; N, 7.27; S, 16.32.

Evaporation to dryness of the filtrates and washings from crude **Ia** and treatment of the residue with absolute ethanol yielded form **Ib** (110 mg), still contaminated with some **Ia**; $[\alpha]_D^{25}$ +11° (*c* 4, 2 N HCl). Ionexchange chromatography on Dowex 50 (in H cycle, elution with 0.36 N HCl) provided a purified sample of **Ib**, that was characterized by its nmr spectrum (Fig. 4b).

Both **Ia** and **Ib** appear on the long column of the amino acid analyzer at an elution volume somewhat less than that of aspartic acid. A ninhydrin constant of 50 was found for **Ia** and 51 for **Ib** (under conditions that give 62 for glutamic acid and also 62 for alanine). On tlc, R_f 0.12 was found for both forms in the system *n*-butanol-acetic acid-water (4:1:1).

An additional amount (75 mg) of **I**, mostly **Ia**, was secured from the third fraction of the elution.

For nmr spectra, samples (30~40 mg) of forms **Ia** and **Ib** were dissolved in 2 N DCl (0.4 ml), evaporated to dryness with a stream of N_2, and redissolved in N DCl (0.4 ml). The spectra were recorded on a Varian A60 instrument.

Acknowledgment

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* No further increase in the amount of **I** was observed after two days.

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